# Beyond Microbiota and Colorectal Cancer: A Critical Review

Dissertação | Revisão Bibliográfica

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## Abbreviations List

APC – adenomatous polyposis coli

BFT – *Bacterioides fragilis* toxin

BMI – body mass index

CEC – colon epithelial cells

CRC – colorectal cancer

FOBT – fecal occult blood test

IBD - inflammatory bowel disease

MIN – multiple intestinal neoplasia

WHO – world health organization

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# Abstract

**Introduction:** Cancer is known to be a major cause of morbidity and mortality, making it vital to deepen cancer research. Colorectal cancer is one of the most common cancers and represents the fourth most common cause of death from cancer worldwide. It is a multifactorial disease associated with environmental exposure, DNA mutations, lifestyle, inflammation and recently, microbiota.

**Aim:** The aim of this review is to discuss the most recent scientific advances in the establishment of a causality link between intestinal microbiota and colorectal cancer, as well as explore the underlying physiopathological mechanisms and theories. It will also be discussed the clinical relevance of the subject.

**Results:** Most of the studies corroborate a relationship between the microbiota changes and tumorigenesis. However, the existence of a causal relation has not yet been fully clarified. More and more evidence suggests a collective and multifactorial role of the microbiome. Nevertheless, some microorganisms appear to play a prominent role, such as *Fusobacterium nucleatum*, *Escherichia coli* and *Enteroxigenic Bacteroides fragilis*. There has been increasing interest in the clinical potentials of this association, namely by microbiome modulation and creation of potential screening biomarkers.

**Conclusions:** There is solid evidence of the relationship between microbiome and colorectal cancer. However, it's not entirely clear yet the causal nature of this relationship, as well as the mechanisms and interactions that characterize it to its fullest extent. The most consistently associated microorganism, and therefore with the greatest clinical potential is *Fusobacterium nucleatum.* For now, the clinical application of these findings is not a reality, but the potentialities are huge.



## Key-words

Microbiome, Microbiota, Cancer, Colorectal, Dysbiosis

## Resumo

**Introdução:** O cancro constitui uma das principais causas de morbilidade e mortalidade, sendo por isso crucial aprofundar a investigação neste campo. O cancro colo-rectal é um dos cancros mais comuns e representa a quarta causa mais comum de morte por cancro em todo o mundo. É uma doença multifatorial associada a exposição ambiental, mutações genéticas, estilo de vida, processos inflamatórios e recentemente, ao microbioma.

**Objetivos:** O objetivo desta revisão será analisar e discutir os mais recentes avanços científicos no estabelecimento de uma relação de causalidade entre o microbioma intestinal e o cancro colo-rectal, bem como explorar os mecanismos fisiopatológicos subjacentes e teorias propostas. Esta dissertação procurará também abordar a relevância clínica da questão estudada.

**Desenvolvimento:** A maioria dos estudos corrobora uma relação entre alterações da microbiota e os processos de tumorigénese, no entanto ainda não está totalmente esclarecida a existência de uma relação causal. Cada vez mais a evidência sugere um papel coletivo e multifatorial do microbioma, no entanto alguns microrganismos aparentam ter um papel de destaque, tais como *Fusobacterium nucleatum, Escherichia coli* e *Enteroxigenic Bacteroides fragilis.* Tem havido um interesse crescente na investigação das potencialidades clínicas desta associação, nomeadamente a nível de modulação da microbiota e criação de possíveis biomarcadores de rastreio.

**Conclusões:** Há evidência da relação entre o microbioma e o cancro colo-rectal, mas ainda não está inteiramente esclarecida a natureza causal da relação, assim como os mecanismos e interações que a caracterizam em toda a sua extensão. O microrganismo mais consistentemente associado e com maior potencial de aplicação clínica é o *Fusobacterium nucleatum*. Para já, a possibilidade de aplicação clínica destas descobertas ainda não é uma realidade, no entanto as potencialidades desta área são amplas.



## Palavras-chave

Microbioma, Microbiota, Cancro, Colorretal, Disbiose

# Background

## Colorectal cancer – Impact and carcinogenesis

Colorectal cancer is defined as a carcinoma, most commonly adenocarcinoma, in the colon or rectum. (1) It is the third most common cancer diagnosed in both men and women in the United States, representing 10% of the global cancer incidence burden. (1, 2) It is the fourth most common cause of death from cancer worldwide.

The incidence and death rates for colorectal cancer increase with age, being that 90% of new cases and 93% of deaths occur in people older than 50 years. (3) More than 65% of the new cases occur in developed countries. (1)

In Portugal, colorectal cancer is the third most common cancer in men and the second most common in women. According to the most recent data (2014), the mortality rate for colon cancer was 25.8% and for rectal cancer was 10.3%. There is an asymmetry regarding incidence and mortality, comparing the coastal area with the interior, being that the latter has higher incidence and mortality rates. The mortality rate is also lower in the urban centers. (4)

The exact etiology of colorectal cancer is still unknown, but it has been linked with genetic, epigenetic and environmental factors. (5) Most colorectal cancer cases are sporadic (70-80%), but it can have a hereditary component (20-30%), due to some susceptibility syndromes, such as Human Non-polyposis Colon Cancer and Familiar Adenomatous Polyposis. A small part of CRC cases (1 – 2 %) can arise as a consequence of inflammatory bowel diseases. (6)

Colorectal cancer is a heterogeneous disease in terms of both molecular and morphologic carcinogenesis pathways. Three molecular carcinogenesis pathways are generally accepted: the chromosomal instability, microsatellite instability and CpG island methylator phenotype or epigenetic instability pathway. (7) In terms of morphology, it's known that most colorectal carcinomas develop through an adenoma-carcinoma sequence, whether about 10 – 20 % of carcinomas may develop via the serrated pathway, a different sequence of morphological changes. (6)

The chromosomal instability pathway is found in about 85% of sporadic colorectal cancers and includes activation of oncogenes like KRAS and the inactivation of tumor suppressor genes such as APC and p53. (1, 5) The microsatellite instability pathway occurs in about 15% of sporadic colorectal cancers, due to an incompetent DNA mismatch repair system, as a result of mutation, deletion or promoter methylation of some MMR genes, such as MLH1, MSH2, MSH6 and PMS2 that codify for the MMR proteins. (6) The epigenetic instability pathway is characterized by

hypermethylation of numerous promoter CpG island loci and consequent inactivation of tumor suppressor genes or tumor-related genes. (7)

The classical adenoma-carcinoma sequence begins with conventional adenomas, including tubular or tubulovillous adenomas, in which the accumulation of genetic and epigenetic alterations leads to epithelial dysplasia and progressive evolution to carcinoma. (1, 5, 7) This pathway is driven by chromosomal instability pathway or microsatellite instability pathway and it usually takes 10 to 15 years until overt cancer. (2) The serrated pathway begins with hyperplastic polyps, sessile or traditional serrated adenomas and has epigenetic instability as its initial driving pathway and microsatellite instability pathway in a secondary role. (7)

Although the triggers leading to this onset of DNA mutations and molecular alterations are not fully understood, it is known that colorectal cancer is mainly a “lifestyle disease”. (1) In most cases, environmental factors such as dietary composition, lack of physical activity, overweight, cigarette smoking and heavy alcohol consumption play a vital role in an individual’s risk of colorectal cancer. (8, 9) This environmental impact is supported by many migrants’ studies. Among migrants from low-risk to high-risk countries, incidence rates of colorectal cancer tend to increase toward those of the host country. (8)

Over the past decade, there has been increasing experimental evidence linking gut microbiome and colorectal cancer, as it will be approached further in this essay. (9)

Therefore, colorectal cancer is one of the major cancers for which modifiable causes may be identified and possibly prevented. A better understanding of the mechanisms involved and interactions between the environmental factors is crucial in the quest for CRC prevention and screening.

## Overview of the gut microbiome

Most of the human epithelial surfaces host a variety of microorganisms, mainly bacteria, that outnumber the human somatic cells by at least a magnitude of 10. However, the greatest number of bacterial cells reside in the digestive tract. The microbiome, defined as the collective microflora genetic material, is 150 times larger than the human genome. (10)

In the gastrointestinal tract, quantitative and qualitative variations are seen in the different sites from mouth to rectum, due to host factors (pH, transit time, bile acids, digestive enzymes, and mucus), nonhost factors (nutrients, medication, and environmental factors) and bacterial factors (adhesion capacity, enzymes, and metabolic capacity). (11) The large intestine contains 70% of the human microorganisms, approximately $10^{14}$ bacteria.(12) It provides a good environment for bacterial growth due to an almost neutral pH, reduction of bile salt concentration and pancreatic secretion, associated with a slow transit time. (13)

Evidence based in classical cultivation techniques established that humans were born with a sterile gut. However, 60-80% of the total microbiota cannot be cultivated, and more recent evidence using sequencing methods suggests that there is colonization prior to birth, for example with detection of microbial DNA in premature infants meconium. (11, 14) Then rapidly after birth the exposition expands and there's a diversification and evolution in the composition, with progressive stabilization until approximately the age of 2,5 years, in which we have an adult-like microbiota community. This early-life period of highly instable microbiota is therefore more sensitive to external influences or aggressions.(15) During adulthood the intestinal microflora remains relatively stable, with minor fluctuations. (12, 16) In the elderly, there's a tendency for a decrease in bacterial abundance and diversity, which may prone to disease.(17)

There is a natural interindividual variation caused by genetic characteristics and also age, diet, host immune system, health status and antibiotic use. Therefore, each human being has its own unique microbiota. (10, 18, 19) However, there are some bacterial species commonly found in human gut microbiota, mainly from the phyla *Firmicutes* (30%-50%), *Bacteroidetes* (20%-40%), followed by *Actinobacteria* and *Verrucomicrobia.* (12, 13) In the colon, more than 90% are from phyla *Bacteroidetes* and *Actinobacteria*.(12) Since the colon is free of oxygen, most microbial populations are strictly anaerobic, such as *Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus,* and *Ruminococcus.* (13) Facultative anaerobes like *Lactobacilli, Enterococci, Streptococci* and *Enterobacteriaceae* are also present but represent a minority. (12)

This complex, diverse and dynamic communities of microbiota are known to play a significant role in health. The microbiota participates in digestion and extraction of nutrients, protection against

infection, in the host immune response, drugs metabolism and is also involved in regulation of host metabolism. (19) There is evidence, mainly from animal models, of the microbiota important effects on the structural development and function of gut-associated lymphoid tissues, T cells and B cells. The gut microorganisms also expand the host's range of metabolic capabilities. They participate in the synthesis of micronutrients such as vitamin K, B12 and folic acid, as well as absorption of calcium, magnesium and iron. These bacteria account for a large range of carbohydrate active enzymes which allow them to break down indigestible dietary residues, releasing short chain fatty acids, known to be important for health and immunity. Microbiota can also influence drug metabolism. For example, it was discovered that *Eggerthella lenta* was responsible for inactivating digoxin in a subset of individuals. (19)

We can also divide the microbiota in mucosa-associated and luminal flora, whether the microbes penetrate the mucosal layer or are located in the lumen.(12) The luminal bacteria are less abundant than the mucosa-associated bacteria. (20) Furthermore, when comparing microbiome from stool and mucosal tissue samples, different populations are found. (21) The colonic mucosal communities are adherent to surface-associated polysaccharide matrices and are therefore less affected by hydrodynamic shear forces. These communities rooted to the mucosa interact with the immune system and appear to be more relevant to diseases such as CRC. (5)

## Methods

For this review, a literature search was performed using the PubMed database. The search key terms were (“microbiome" or “microbiota" or "intestinal flora”) and (“colorectal” or “colon” or “rectal” or “rectum”) and (“cancer” or “neoplasm” or “neoplasia”). Only articles published in English and during the period of January 2013 to June 2016 were selected.

The articles of the initial search have been screened for their potential eligibility according to the content of the title and/or abstract. In a second fase, the initially selected papers were full text reviewed, and a final selection was made. Articles considered off-topic, non-relevant or redundant in their results were excluded.

Whenever necessary and relevant, additional papers/documents were included in order to complement the review.

# Microbiota and Colorectal Cancer

## Tumorigenesis and dysbiosis – Is there an association?

One third of cancers worldwide are associated with identified single microbial infections. (22) Concerning CRC, investigation linking the colon commensal microbiota with colon cancer begun in 1969. (23) Since then, the interplay between intestinal microbiota and CRC has been a growing area of research.

CRC is still one of the most prevalent and lethal cancers, and the screening methods available (colonoscopy and FOBT) are either invasive or lack high sensitivity and specificity. (24) The human gut hosts a broad and diverse community of bacteria that plays key roles in the host immunity and metabolism, and possibly in disease. With this in mind, *major* research questions started to emerge, and the first investigation step was comparing the microbiota of healthy individuals with that of colorectal cancer patients.

Several studies used next generation sequencing techniques to compare luminal (fecal samples) and/or mucosa-associated (mucosal biopsy samples or rectal swabs for mucosa-adherent specimens) microbiota of CRC patients and healthy individuals. Many of them consistently found CRC associated dysbiosis, and Table 2 (page 28) sums up the main bacteria involved.

Most colorectal carcinomas develop through an adenoma-carcinoma sequence, so the next step was trying to understand if dysbiosis is only observed in the context of malignancy or if it begins earlier, by the time adenomas appear. Several studies that include microbiome analysis of subjects with adenomas were reviewed and the results are also organized and summed up in Table 1.

The overall bacteria diversity in adenomas is reported in some studies as higher than that of the normal individuals (25, 26). Regarding CRC patients, studies found both lower bacterial population diversity (27-29) and an increasing diversity. (30, 31) Is has been hypothesized that the lower diversity detected in diseased sites is due to a selective enrichment of a reduced number of potentially pathogenic microorganisms due to specific characteristics of the tumor microenvironment, at the expense of many other species, possibly more sensitive to environmental changes. (31) The higher bacterial diversity detected in adenoma patients and in some groups of CRC patients, could be due to the intense irrigation of tumors and polyps, providing more nutrients. (31) Another factor that might be associated with the divergent results in CRC microbiome diversity is the different stage of tumor progression in different studies.

As we can conclude from Tables 1 and 2, the most consistent finding is an enrichment of *Fusobacterium* in CRC patients and in patients with adenomas, both in the gut lumen and mucosa. There is also an enrichment of genus *Porphyromonas, Peptostreptococcus* and the family *Enterococcaceae* in CRC patients compared to healthy controls. Regarding the *phylum* Proteobacteria*,* studies are not completely concordant. Some papers report a Proteobacteria enrichment in the lumen and mucosa of adenoma patients, specially a significantly increase in some genus such as *Pseudomonas*, *Helicobacter* and *Acinetobacter*. On the other hand, for CRC patients, there is a decrease in Proteobacteria abundance in some studies, while other studies show that *Campylobacter and Escherichia coli* were significantly more abundant in CRC cases. (Table 2) *Lactococcus* was also found to be over-represented in the mucosa of adenoma and CRC patients. (Table 1 and 2)

Individual studies discovered some over-represented bacteria listed in Table 1 and 2, for example *Parvimonas micra, Akkermansia muciniphila, Leptotrichia, Methanobacteriales,* but this results were not replicated in more than one study. However, it seems relevant to refer that *Parvimonas micra* showed strong association with CRC across five different cohorts, four of them ethnically different. (32)

Studies also demonstrate a significant reduction in some bacteria. *Bifidobacterium* and *Faecalibacterium* were consistently under-represented in gut lumen and mucosa, both in adenoma and CRC patients. *Lachnospiracae* also showed reduction in adenoma and CRC patients, and there are also reports *of Ruminococcus* reduction, but only in CRC patients. (Table 1 and 2)

One study observed significant over-representation of the phylum *Firmicutes* in adenomas, one of the most abundant phyla in healthy human gut microbiota, as referred before*.* Another study reported under-representation of *Firmicutes* in CRC patients. (Table 1 and 2) It's possible that organisms belonging to the same phylum have different functions in the gut microenvironment and therefore different abundance.

The *Bacteroides* genus was found to be increased in adenoma cases in more than one study, and the majority of studies reported an over-representation of *Bacteroides* in CRC patients. However, one study discovered an under-representation of this genus in stools of CRC cases. *Bacteroides* possibly has both beneficial and detrimental effects on host, through their colitogenic or probiotic potential. (33)

There were also many discording results regarding some bacteria, such as *Prevotella*, *Blautia* and *Roseburia,* with studies reporting under or over-representation of this genus.

These differences and results that don't replicate between studies seem to reveal that there is a diversified pattern in human CRC microbiome community. This makes sense since there is a natural inter-individual variation in normal gut microbiome, and regional differences must be taken into account. The different stage of tumor progression in different CRC individuals may also account for some part of this variance. Differences in sampling methods (stool sampling or mucosal biopsies), in the experimental approaches and the small sample size of some studies also contribute to these different results. The main goal is to identify within the normal variations, some patterns or specific signature associated with disease states.

The fact that in many studies, the microbiota of individuals with polyps was found to be significantly different to that of controls suggests a possible role of microbiota in the early stages of tumorigenesis, and that the microbiota differences in patients with cancer are not secondary to the cancer itself. However, this finding is not completely consistent between studies, since Zeller *et al* for example, concluded in a metagenomic study that adenomas microbiota composition was almost indistinguishable from healthy controls. (34)

*Bacteria clusters approach*

Some studies tried to address the complexity of the relationship between microbiota and disease by grouping the bacteria in co-abundance groups, based on the idea that the whole community structure can be more informative than individual taxa. (35) Flemer B. *et al.* results showed that individuals with CRC can be stratified into four different groups based on the abundance of bacterial co-occurrence networks. While the individual taxa significantly more abundant in CRC cases were overabundant in only a subset of CRC cases, with the microbial clusters of CRC-associated microbiota, at least one cluster was elevated more than twofold in all but one of the individuals with CRC. Nakatsu *et al* observed that a metacommunity characterized by microbes of oral origin (*Fusobacterium, B. fragilis, Gemella, Peptostreptococcus and Parvimonas)* forming a symbiotic network was associated with CRC. (36)

*Prospective insight with murine models*

Zackular *et al*. used modified murine models (azoxymethane administrated as a mutagen for tumorigenesis induction and dextran sodium sulfate to induce inflammation) and treated them with antibiotics, to see in what way modifications in the microbiota induced by the antibiotics altered the number of tumors. They first realized that the relative abundance of *Lactobacillus* in the beginning of the experiment was inversely proportional to tumor burden in the end. They also

demonstrated that based on the microbiota composition at the beginning of the model they could predict the final number of tumors. So, targeting the gut microbiota in the beginning of dysbiosis is a possible way of improving tumorigenesis. In the authors opinion, microbiota's role in CRC is possibly of polymicrobial nature. (37) Another study inoculated germ-free mice with fecal microbiota from different human subjects, some of them healthy and some with CRC. Then they also induced tumorigenesis using azoxymethane and concluded that the baseline microbiome structure was strongly associated with the final number of tumors, but the cancer status of the human donors was not. Generally, some gram-negative taxa (such as *Bacteroides, Parabacteroides, Akkermansia*) were strongly positively correlated with increased tumor, while members of the gram-positive *Clostridiales* were negatively correlated with tumors. (38)

## Microbiome role in tumorigenesis promotion

### *Bacterial Driver-Passenger Model and Alpha-bug Hypothesis*

One of the main questions investigators have asked is how do bacteria play a role in oncogenesis. Is it driven by multiple species acting collectively? Are there protagonists, or even single microbes capable of promoting cancer by themselves?

Some authors started to come up with theories in an attempt to better understand the role of the bacteria in tumorigenesis.

*Harnold et al* proposed a bacterial counterpart of the genetic "adenoma-carcinoma sequence" model formulated by Fearon and Vogelstein, the driver–passenger model. According to this model, the colonic mucosa of individuals more prone to develop CRC is colonized with certain pathogenic intestinal bacteria. These bacteria drive epithelial DNA damage by persistent inflammation, increasing cell proliferation and/or production of genotoxic substances. This would trigger the subset of pre-malignant lesions and the subsequent accumulation of mutations, leading to the initiation of carcinogenesis. There are designed "bacterial drivers". (39)

Then tumorigenesis, with rupturing and bleeding of the cancerous tissue, induces local intestinal alterations like changes in colonic barrier permeability and cellular metabolism. This changes would favor the proliferation of not only opportunistic bacteria, but maybe also commensal or probiotic bacteria, being this bacteria designated the "bacterial passengers". They hipothyse that bacterial drivers may disappear from cancerous tissue as they are outcompeted by passenger bacteria with a growth advantage in the new tumoral microenvironment. (39) So, according to this theory, bacterial drivers and passengers have distinct temporal associations and probably distinct roles in tumorigenesis. (39)

Another model, the "alpha-bug" hypothesis, proposed by Sears and Pardoll, relates to this one in the sense that the authors suggest that some microorganisms, designed "alpha-bugs" are directly pro-oncogenic, by their ability to modify the mucosal immune response and alter the colonic bacterial community. (22) By this mechanisms, the alpha bugs together with helper bacteria, possibly "crowd out" some beneficial species. This "alpha bugs" and the helper bacteria correspond to the driver bacteria, in the driver-passenger model. (39) However, according to this hypothesis, drivers persistently colonize the developing tumor and are not outcompeted by other bacteria. (22) This theory arose from their studies of enteroxigenic *Bacteroides fragilis*, and this microorganism is the protagonist of this model. (22)

The authors of the driver-passenger model propose as possible main bacterial-drivers pathogenic members of Bacteroides, such as enteroxigenic *Bacteroides fragilis* or the family *Enterobacteriaceae*, highlighting the uncertainty of this hypothesis. For main bacterial passengers are proposed the pathogens *Fusobacterium* or *Streptococcus spp*. and commensals like members of the *Coriobacteriaceae* family. (39)

After this proposals many studies have emerged. Different studies found an enrichment of *Fusobacterium* in CRC but there's also a study reporting over-representation in adenomas, noting that there was higher abundance in tumor that in adenoma samples. (31) This suggests a possible active involvement also in early CRC development. According to the alpha-bug theory, *Fusobacterium* could possibly be considered an alpha-bug. This information isn't also incompatible with the driver-passenger theory since, as the authors highlight, this model does not exclude the involvement of some of the bacterial passengers in the beginning of pathogenic alterations. If the new tumoral microenvironmental provides preferable conditions for this bacteria, then they will continue to grow and participate in CRC progression. (39) Therefore, the bacterial driver-passenger model is apparently more complete, including the alpha-bug definition, but also proposing new hypothesis.

In Tables 1 and 2 we can see that many of the bacteria reported as over and underrepresented in adenomas were not reported in CRC, and vice versa. For example, *Porphyromonas*, and *Peptostreptococcus* were not mentioned in any of the studies with adenomas, but were reported as over-represented in some of the studies with CRC samples. According to the driver-passenger theory, these bacteria could be possible bacteria-passengers. Mira-Pascual *et al* hypothysed that the family *Enterococcaceae* could be a possible driver, since besides being present in all samples, its proportion was higher in the adenoma group, comparing to healthy controls and adenocarcinoma. (31) Chen *et al* reported increased abundance of the family *Coriobacteriaceae* in CRC patients; besides, none of the adenoma studies analyzed reported significant alterations in *Coriobacteriaceae* abundance (Table 1 and 2). These findings support *Harnold et al* proposal that the family *Coriobacteriaceae* could be a possible passenger. *Lactobacillus* is enriched in adenomas and reduced in CRC samples, fitting in the bacteria-driver profile*. Enterobacteriaceae* is only reported to be enriched in adenoma samples, which corroborates the authors hypothesis on this family being possible bacteria-drivers (Table 1 and 2).

Geng *et al* proposed that in Han Chinese population, *Roseburia,* which is overrepresented at tumor sites, could be a passenger bacteria, whereas *Microbacterium* and *Anoxybacillus,* overrepresented only in adjacent non-malignant tissue, could be possible drivers. (30)

The fact that there are some bacteria (*Lachnospiracae* family, *Faecalibacterium, Bifidobacterium*) down-regulated already in the adenoma phase and consistently diminished in CRC samples compared to normal controls (Table 1 and 2), is not contemplated or explained in this model.

Concerning the *Proteobacteria* phylum, some bacteria like *Escherichia coli* and *Campylobacter* were only reported to be overexpressed in CRC samples (Table 1 and 2) and could be possible passengers, while *Pseudomonas* could be proposed as a possible driver since some studies found and enrichment in adenomas and there aren't similar reports in CRC patients (Table 1 and 2).

Lu *et al*. when comparing adenomatous and adjacent non-adenoma tissues found similar microbiota structure. This might mean that the driver-passenger hypothesis may not be so relevant to the precancerous colon lesions. (25)

## Mechanisms of interaction with the host

The studies that have been presented so far have focused on identifying the taxonomic classes associated with CRC. Furthermore, it's important to understand if and what bacterial products may have an impact in health and disease, and explore the potential bacterial interactions.

Zackular *et al* results suggest that CRC is a polymicrobial disease, under the influence of many microorganisms and interconnected mechanisms. When they analyzed each patient individually, they rarely identified significant enrichment of every bacterial population studied. However, using the relative abundance data for a selected microbial panel, they managed to accurately classify the subjects as healthy, adenoma or carcinoma cases. (40) This suggests that along with some individual protagonists, microbiota may also have a strong collective role involving complex and interconnected mechanisms.

Chen *et al* postulate that bacteria and it's components function by directly interacting with the host or in an indirect way, through co-metabolism or metabolic exchange with the host. (28) According to their results, the predominant phylotypes in lumen of CRC patients (*Erysipelotrichaceae, Prevotellaceae, and Coriobacteriaceae*) have all been associated with metabolic disorders or energy metabolism and linked with obesity and high-fat diets. They also compared fecal and mucosal samples and found higher *Firmicutes* in gut lumen and higher *Proteobacteria* in mucosa. *Firmicutes* has been demonstrated to enhance energy harvest from diet and *Proteobacteria* potentially exhibits direct interaction with intestinal cells. (28)

Zeller *et al.* found a global metabolic shift between CRC cases and controls, from utilization mainly of dietary fiber in the healthy participants, to predominant host-derived energy sources in CRC. Host cell-derived metabolites like amino acids were more abundant in the tumor environment, and data suggested an increased capacity of amino acids uptake and metabolism by the microbiota, via the putrefaction pathway. The metabolites from this pathway include polyamins, which if accumulated intracellularly can promote tumorigenesis. This finding raises the question of whether this increased putrefaction is a tumoral consequence or has a causal role in tumorigenesis. They also observed an enrichment in LPS metabolism, which has pro-inflammatory potential, triggering an inflammatory signaling cascade. (34)

Sinha *et al* found significant microbiome-metabolite correlations in human feces, and also microbiome-metabolite differences between CRC cases and controls. Fecal samples from CRC patients were associated with significantly under-representation of *Clostridia*, *Lachnospiraceae,* p-aminobenzoate and conjugated linoleate, and with higher levels of *Fusobacterium, Porphyromonas*, palmitoyl-sphingomyelin and p-hydroxy-benzaldehyde. Palmitoyl-

sphingomyelin correlated directly with abundances of *Enterobacteriaceae, Actinobacteria* and *Firmicutes.* However, it isn't known yet how this metabolites impact CRC risk. (41)

Nugent *et al* also found that the metabolome differed significantly between adenoma patients and healthy individuals. It is noteworthy an increase of the inflammatory metabolite Prostaglandin E2 and a decrease in two antioxidant-related metabolites, 5-oxoproline and diketogulonic acid. This suggests the importance of inflammation and possible role of oxidative stress in adenoma development. (42)

Ohigashi *et al* detected lower concentrations of short chain fatty acids in feces of CRC individuals, and an associated increase in pH. More precisely, three types of organic acids (acetic acid, propionic acid and butyric acid), usually the most abundant in the gut, were reduced. (43) Short chain fatty acids are important final products of bacterial carbohydrate fermentation in the gut. Butyrate in particular is thought to be important in maintenance of a healthy intestinal environment. It's considered to be the preferred energy substrate for the colonocytes, and apparently stimulates a physiologic pattern of cell proliferation and suppresses tumor cells proliferation in the colonic crypts. (44, 45) It also participates in the maintenance of intestinal acidity, prevention of toxin absorption and promotion of cancer apoptosis. (43) According to Hold *et al*, some of the main butyrate-producing bacteria are *Roseburia intestinalis*, *Faecalibacterium prausnitzii* and *Eubacterium hallii.* (46) In Tables 1 and 2 we can see that *Faecalibacterium* and *Roseburia* were found diminished in CRC/adenoma cases in some studies. In adenoma cases, fecal short chain fatty acids and pH were intermediate between normal individuals and CRC cases, and there were no differences detected between different CRC stages. This suggests that these variations are not consequent to the cancer itself. (43) Baxter *et al.* found a negative correlation between the number of tumors and butyrate production capacity. (38)

It was also found a positive correlation between tumor count and mucin degradation. Disruption of the mucosal barrier integrity by mucin degradation could possibly lead to increased inflammation. (38)

This are some of the main mechanisms that are thought to take part in promotion of carcinogenesis by bacterial populations. However, there's a much wider range of possible interactions and mechanisms studied, and mainly a lot of questions to answer.

## Role of individual intestinal bacteria

Besides the belief that the microbiota influence in carcinogenesis is collective and polymicrobial, studies have identified some species consistently increased in the tumor microenvironment and there is growing evidence of a protagonist role of this species in tumorigenesis.

### *Fusobacterium nucleatum*

*Fusobacterium* species are gram-negative, anaerobic bacteria from bacteroidaceae family. (47) As we can see from Tables 1 and 2, there is accumulating evidence linking *Fusobacterium* with colorectal cancer. *Fusobacterium nucleatum* is the most common microorganism in the subgingival biofilm. It is considered a relevant pro-inflammatory factor in the oral cavity and is involved in periodontal diseases. (48) It has been consistently found in adenoma and tumoral tissues, leading to the thought that maybe it also participates in gastrointestinal diseases, in particular colorectal tumorigenesis.

McCoy *et al,* besides finding increased abundance of *Fusobacterium* in adenoma mucosa samples, found a positive correlation between local cytokine gene expression and *Fusobacterium* abundance, in particular for TNF-α and IL-10.(49) This leads us to speculate that possibly *Fusobacterium* conditions mucosal inflammation and therefore contributes to the initial tumorigenesis. Kostic *et al.* conducted an experiment using min[1] mice and found that *Fusobacterium* accelerated the onset of colonic tumors and recruited tumor-infiltrating myeloid cells, with immune suppressive activity. Tumors from the min mice exposed to *Fusobacterium* had a pro-inflammatory expression signature, characterized by an increase in tumor-associated neutrophils, tumor-associated macrophages and dendritic cells including $CD103^+$regulatory dendritic cells. This subtype of dendritic cells expressing CD103 integrin can promote the expansion of $Foxp3^+$regulatory T cells, a $CD4^+$ T cell subset that inhibits cytotoxic and effector T cells, therefore diminishing anti-tumor immunity. This pro-inflammatory environment could help tumor progression. (50) Another possible competitive advantage could be due to the fact that *Fusobacterium nucleatum* doesn't have the need to compete for glucose, a substrate used by the

[1] Multiple intestinal neoplasia (Min) mouse strain is considered the classic murine model of colon tumorigenesis and is heterozygous for the adenomatosis polyposis coli (APC) allele. Mutations in APC occur in nearly all human colon cancers and are the characteristic molecular defect in Familial Adenomatosis Polyposis syndrome. (22)

tumoral cells. Besides that, *F. nucleatum* has a rudimentary electron transport chain, which allows it to survive in the hypoxic tumoral microenvironment. (50)

Mima *et al* tried to test the hypothesis that the amount of *F. nucleatum* in colorectal carcinoma tissue is inversely correlated with the density of T cells in tumor tissue, knowing that higher levels of T cell infiltrates in colorectal carcinoma have been associated with better outcomes. They found an inverse association between the amount of *F. nucleatum* and $CD3^+$ T cells density. (51)

Tahara *et al* results showed that a higher level of *Fusobacterium* was related with a molecularly distinct type of cancer, characterized by high rates of CpG island methylation microsatellite instability. With this results they suggest that the higher *Fusobacterium* levels in CRC are not just an epiphenomenon of the cancer itself. The fact that *Fusobacterium* levels were also high in the adjacent normal tissues corroborates this idea. (52) A recent study also demonstrated that the abundance of *Fusobacteria* was similar between tubular adenomas and sessil serrated adenomas, suggesting that *Fusobacteria* could possibly influence both tumorigenesis pathways. (53) It was observed a higher presence of *F. nucleatum* in tumors than in polyps in both fecal and mucosal samples. It's tempting to speculate that a higher presence of *Fusobacterium* would be representative of high risk of CRC, and could also represent a potential biomarker of carcinogenesis development. (31) A recent study showed that the amount of *F. nucleatum* DNA in colorectal cancer tissue is positively associated with shorter survival. This findings open doors for investigations exploring *F.nucleatum* as a potential prognostic biomarker. (54)

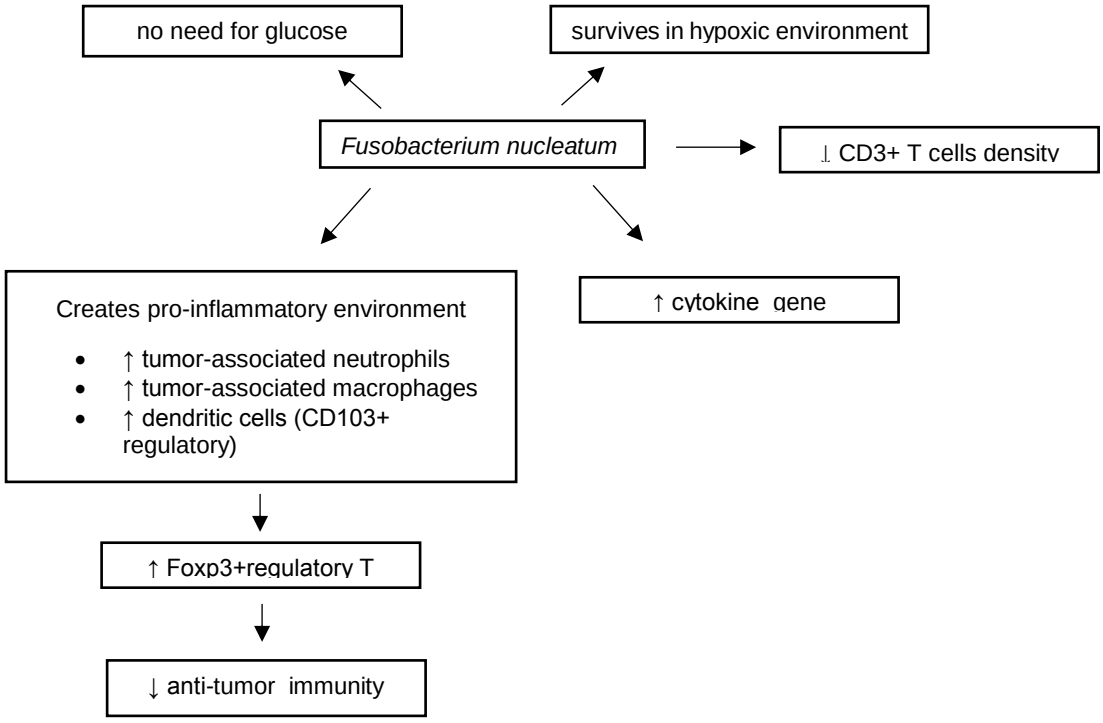


*Figure 1. Fusobacterium nucleatum* - Mechanisms of tumorigenesis

*Escherichia coli*

*E.coli* is a commensal microorganism of the human intestinal microbiota. However, some pathogenic strains developed the ability to induce chronic inflammation and/or produce toxins. (55) Four phylogroups are known: A, B1, B2 and D. Commensal strains usually belong to the A or B1 phylogenetic group and pathogenic strains normally belong to B2 or D phylogroup. Pathogenic groups have the ability to produce more virulent factors than commensal strains. The genotype B2 harbors the *pks* genomic islands. This islands lead to the production of a genotoxin, colibactin. (56) Infection by *pks* positive *E. coli* strains leads to production of oxygen species, pro-inflammatory cytokines and protease secretion. These can trigger DNA-double strand breaks. (57)

These bacteria are part of the *Enterobacteriaceae* family, and bacteria from this family produce proteins called bacteriocins. Kohoutuva *et al* found a significant higher production of bacteriocins in patients with advanced adenoma when compared to patients with non-advanced adenoma, and also higher production of bacteriocins with more advanced CRC stages. They also found significantly higher abundance of *E.Coli* phylogroup D in CRC patients and associated the B2 phylogroup with right-sided CRC. (57)

Arthur *et al* developed an experiment relating inflammation with cancer. First, they concluded that colitis-susceptible $IL10^{-/-}$ mouse strain developed a different microbiota comparing to wild-type controls, suggesting that inflammation alters the microbiota. The luminal microbiota of colitis-susceptible mice exhibited a 100-fold increase in *E. coli.* Through the addition of a colon-specific carcinogen (azoxymethane) they were able to conclude that this microbial shift was related to inflammation and not with cancer itself. In a second phase, they decided to create two groups of colitis-susceptible mouse with azoxymethane, and one group was mono-associated with *E. coli* whereas the other group was colonized with *Enterococcus faecalis.* 80% of *E. coli* mono-associated mice developed adenocarcinomas, while *E. faecalis* mono-associated mice rarely developed tumors. They also investigated the presence of *pks* + *E. coli* in IBD, CRC patients and controls, and found a significantly higher percentage in inflammatory bowel disease and CRC patients, suggesting a possible association. These investigators went deeper in the investigation and analyzed the infection of a rat intestinal epithelial cell line with a *pks* deficient *E. coli* strain, and compared with infection of the same cell line with a normal *pks* + *E. coli* strain. Infection with normal *E.Coli* induced DNA damage in 30% of cells, whereas infection with *pks* deficient *E.Coli* induced DNA damage in less than 5%. Moreover, when infecting germ-free and colitis-susceptible mice (with and without azoxymethane treatment) with *pks* deficient *E. coli* strain and normal *pks* + *E. coli* strain, they found decreased tumor multiplicity and invasion mice with *pks* deficient *E. coli* strain, without alterations in intestinal inflammation. (58)

*Bonnet et al.* found a significant relationship between poor prognostic factors for colon cancer and colonization of the mucosa by *E. coli.* Also, it was observed that pathogenic cyclomodulin-positive *E. coli* strains were more prevalent on the mucosa of patients with more advanced colon cancer stages. They also inoculated min and wild-type mice with a colon cancer–associated *E. coli* strain and verified a significant increase in the size and number of tumors compared to controls only in min mice. This results can also suggest that tumor development induced by infection with *E.coli* requires a first hit, such as an APC mutation. (55)


*Figure 2. E. Coli* - possible mechanism of patogeny

Enteroxigenic *Bacteroides fragilis*

*Bacteroides fragilis* is a common colonizer of the human colon, but represents a minority of the bacterial community. Enteroxigenic *B.fragilis* is a molecular class of *B. fragilis* characterized by the secretion of a metalloprotease toxin termed the *B. fragilis* toxin. This bacteria appears to behave either as a pathogen or a commensal. It is associated with diarrheal illnesses, but asymptomatic colonization is also common. (22)

Studies investigating the mechanism of action of BFT showed that the toxin stimulates cleavage of E-cadherin, a structural protein comprising the *zonula adherens* of CECs and that acts as a suppressor of colon tumorigenesis. Cleavage of E-cadherin by BFT leads to increased colonic permeability and exposition of the submucosa to luminal bacterial antigens, possibly inducting colon inflammation. (22) This cleavage also leads to activation of β-catenin nuclear signaling, with consequent activation of Wnt signaling, leading to cell proliferation. In addition, BFT also stimulates synthesis and secretion of pro-inflammatory cytokines (IL-8 and TNF-a) through activation of nuclear factor-ƙB signaling. (22) A study analyzed the T cell-dependent mucosal immune responses in ETBF-colonized mice and compared it with controls. ETBF rapidly induced activation of Stat3, a transcription factor that mediates, in part, T cell lineage development and is also regulator of oncogenesis. Stat3 activation is required for the induction of Th17 immune responses, whose effector cytokine is IL-17. Two populations of T cells, were identified to be producing IL-17 in the colon mucosa of ETBF-colonized mice but not in controls. They also further blocked IL-17and observed that it significantly inhibited ETBF-induced colon tumorigenesis. (22) So ETBF is thought to alter CEC and mucosal immune function to promote oncogenic mucosal events. It may also promote DNA damage either through direct action of the toxin on CEC or reactive oxygen species released by inflammatory cells. (22)

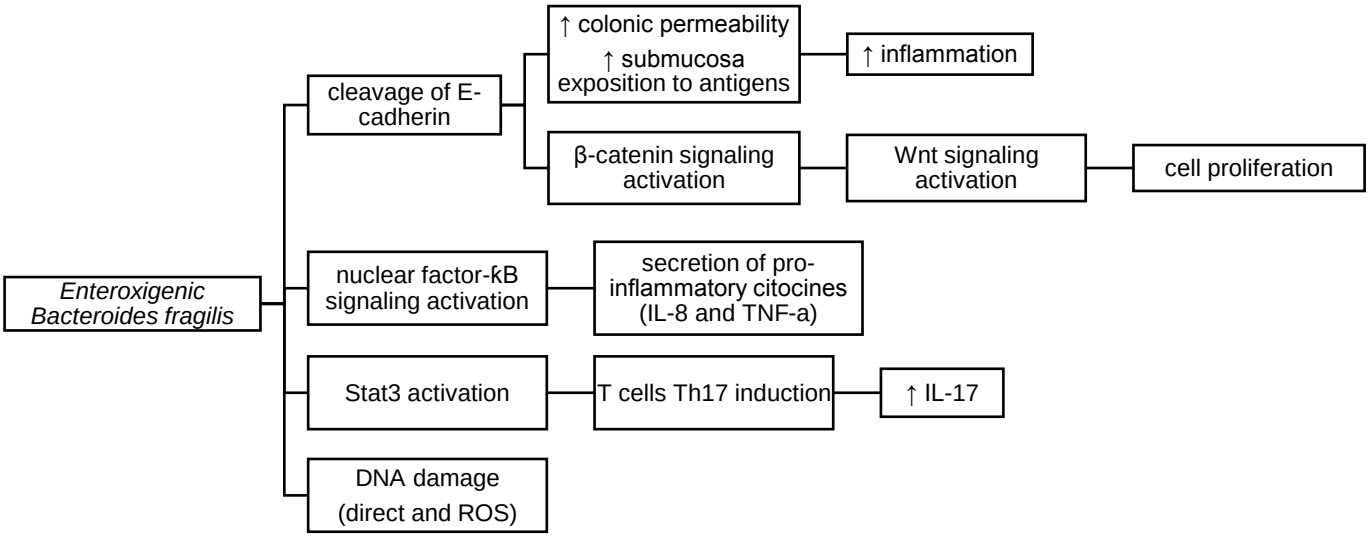


*Figure 3.* Enteroxigenic *Bacteroides fragilis* – mechanisms of tumorigenesis

## Biofilms

Biofilms consist in aggregations of microbial communities encased in a matrix, that adhere and invade the colonic mucus layer and therefore, directly contact with mucosal epithelial cells. (59) They are found in numerous chronic mucosal diseases. It's been a recent area of research whether these microbial formations play a role in CRC initiation. Dejea *et al* showed that right sided CRC is biofilm-positive in 87%, and 100% of adenomas were also biofilm positive. On the contrary, tumors located in the transverse and descending colon displayed biofilms in 13% and 0% of CRCs and adenomas, respectively. This points out the possibility of biofilms being a distinct characteristic of proximal tumors. (59) All biofilm-covered CRCs showed bacterial invasion into the tumor, opposite to the biofilm-negative tumors. The predominant bacteria featuring the biofilms were *Bacteroidetes* and *Firmicutes*, and less commonly *Fusobacteria* and *Gamma-proteobacteria.* Biofilms were also present in normal colon tissue from the tumor patients. Dejea *et al* also showed it was associated with reduced or altered E-cadherin, enhanced IL-6 and with Stat3 activation. This suggests that biofilms might increase epithelial permeability and promote procarcinogenic tissue inflammation. (59)

Johnson *et al* tried to better understand the metabolic influence of biofilms on colon tissues. They found an enrichment of a polyamine ($N^1$, $N^{12}$-diacetylspermine) in both biofilm positive cancer and normal tissues, comparing with biofilm-negative tumor tissues and healthy controls. The authors propose a model where bacterial polyamine metabolites act synergistically to promote biofilm formation and enhance cellular proliferation, possibly leading to oncogenic transformation in colonic epithelial cells. (60)

The investigation concerning biofilms and their potentially pro-oncogenic role is still beginning to grow and there aren't yet many answers concerning this topic.

## Beneficial roles of bacteria

Many authors hypothesized that certain bacteria may have a role in protection against pathogens and possibly prevent the progression of cancer. (28)

Feng *et al* observed that some of the control-enriched species were lactic acid-producing bacteria *Bifidobacterium animalis*, *Streptococcus mutans* and *S. thermophilus.* Lactic acid participates in gut acidification and inhibits intestinal amino acid degradation. It was also reported to accelerate colon epithelial cell turnover in mice. There is evidence that advanced colorectal adenoma or carcinoma patients are deficient in lactic acid-producing commensals such as *Bifidobacterium,* that could potentiate daily epithelial renewal and inhibit potential pathogens. (61) *Lactococcus*, also a lactic acid-producing bacteria, were over-represented in CRC patients besides playing a probiotic role in colon. The question that remains is whether these solely benefit from the CRC microenvironment or if they also play an active part in disease. (62)

Short chain fatty acids are important microbial metabolites and butyrate has been shown to have substantial anti-tumorigenic properties. (44) As approached before in this review, butyrate is thought to be important in the maintenance of a healthy intestinal environment, participating in several benefic and antitumoral processes. Some of the main butyrate-producing bacteria (*Roseburia intestinalis*, *Faecalibacterium prausnitzii*) were found diminished in CRC/adenoma cases in some studies. This loss of short chain fatty acids producing bacteria populations is likely to play a synergistic role in potentiating tumorigenesis. (40)

*Lactobacillus spp.* interacts with the host by binding to human mucus and they are currently used as probiotics. It is not yet understood if the effect is direct (through immune modulation, for example) or indirect (via alteration of the intestinal microbiota) (48)

## Clinical Relevance

Zackular *et al*. identified a panel of bacterial populations that could indicate both the progression from healthy tissue to adenoma and the progression from adenoma to carcinoma, and created a screening model combining BMI, FOBT, and the microbiome data. This model provided excellent discriminatory ability. They also compared the microbiome test with the FOBT, and assessed that the likelihood ratio of a positive FOBT was lower than the likelihood ratio of a positive microbiome test. For better understanding, they explained that for a 65 years old person with a positive FOBT, there was a 1 in 15 chance of having an adenoma. This contrasts with 1 in 9 chance using a positive microbiome test in the same 65-year old. It was concluded that the sensitivity of the microbiome test was greater than the sensitivity of the FOBT. (40)

George Zeller *et al* used metagenomics to explore microbiota potential for CRC detection, hypothesizing that a combination of marker species could be used to improve screening. They selected the four most discriminative species, (two *Fusobacterium* species, *Porphyromonas asaccharolytica* and *Peptostreptococcus stomatis)* enriched in CRC patients. This metagenomic classifier proved to be slightly better than FOBT. They also combined the two tests and obtained a sensitivity 45% higher than FOBT alone. The authors then assessed for external validation, applying the classifier in cohorts from different countries. They concluded that high accuracy detection was still possible even with cohort differences. It was also concluded this classifier has potential for early detection, since the sensitivity was similar for early-stage and late-stage CRC. These markers were also tested in IBD patients, and the most discriminative markers were all significantly higher in CRC, proving its specificity for CRC. (34) The future application of this markers in population screening relies on the development of cost-effective methods. With this in mind, Zeller *et al* tested an alternative 16S sequencing classifier for CRC, and it accomplished almost as good an accuracy as the metagenomic model. (34)

A recent study tested the effect of probiotic *Lactobacillus salivarius* REN[2] in a 1,2-dimethyl hydrazine (DMH - induces colon carcinogenesis) induced mice model. Injection with DMH significantly altered the gut microbiota, while *Lactobacillus salivarius* REN promoted some reversion of this alterations. With carcinogenesis induction, the amount of *Ruminococcus* and *Clostridiales* increased, while *Prevotella* sp decreased. The probiotic bacteria reduced the amount of *Ruminococcus, Clostridiales* and *Bacteroides dorei,* and increased the amount of *Prevotella*.

[2] L. salivarius REN is a novel strain isolated from the fecal samples of Chinese centenarians (63)

These results suggest that some bacteria are capable of modulating other bacteria populations, and could possibly be used as a way to prevent carcinogenesis. (64)

Baxter *et al* found a positive correlation between tumor count and mucin degradation, which would be interesting to confirm with further experiments since blockage of mucin degradation could be used as a future therapeutic target in tumorigenesis prevention or delay. (38)

A recent study concluded that probiotics *Clostridium butyricum* and *Bacillus subtilis* inhibited the growth of CRC cells in mice both *in vitro* and *in vivo*, by promoting apoptosis and triggering cell cycle arrest. The anticancer effects seem to relate with the production of antiproliferative compounds (butyrate, bacitracin), suppression of inflammation and immune modulation. (65) The next step is to investigate if these findings replicate in human studies.

Researchers are also exploring the possibility that the negative effects of *Fusobacterium* might be regulated by therapeutic interventions. Recently, Kumar *et al*. identified a set of target proteins suggested to be crucial for survival and pathogenicity of the bacteria. This finding can lead to future development of drugs to target these proteins and therefore diminish *Fusobacterium* effect on cancer progression. (66) A recent Chinese study demonstrated that Berberine, a component of the Chinese herb *Coptis chinensis,* reversed the *F. nucleatum*-mediated increase in opportunistic pathogens and also inhibited some tumorigenesis-related pathways. (67)

It's also plausible to speculate that short chain fatty acids producing bacteria, as other beneficial bacteria, could integrate possible health biomarkers in CRC prevention.

## Conclusion and future directions

Overall, the relation between microbiome and colorectal cancer is a growing area of research, complex, broad and continually developing. There is much that we already know, but there are many more questions to answer.

There is sustained evidence that microbiome is indeed related to colorectal cancer, and there has been a lot of research exploring and comparing the composition of clinically different populations. However, it's not fully understood if there is a causal relation, if the changes are a cause or consequence of cancer itself, or a bit of both. Further research is needed to better understand the vast and complex mechanisms and interactions involved. Another challenge is to find a microbiome signature associated with colorectal cancer, given the great interindividual variety of microbiome populations. The most consistently associated microorganism so far, and therefore the one showing most applicability potential is *Fusobacterium nucleatum.* It's also important to highlight and further explore the fact that besides the potential pathogenic role, some bacterial species may also play a protective role in colorectal cancer.

This area of research has a promising potential in improving prevention, diagnosis and even treatment of colorectal cancer. Modulation of the microbial flora could possibly be used in cancer prevention. The actual screening and diagnostic methods are far from ideal: FOBT lacks sensitivity and specificity and colonoscopy is invasive and expensive. Microbiota can possibly be used to create screening biomarkers and therefore improve screening and diagnosis. It may also contribute to more directed and personalized treatments. The possibilities are immeasurable, but further prospective human studies in large populations are needed to answer the unknown questions and provide better knowledge about this complex but ultimately fascinating area.

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# Appendices

## Table 1

| Phylum | Microbiota | Change in adenomas compared to control | Type of sample | Reference |
|---|---|---|---|---|
| **Firmicutes** | | reduced | mucosa | (25) |
| | *Enterococcaceae* | increased | feces and mucosa | (31) |
| | *Lactobacillus* | increased | mucosa | (26) |
| | *Lactococcus* | increased | mucosa | (25) |
| | *Ruminococcaceae* | increased | feces | (40) |
| | *Faecalibacterium* | reduced | Mucosa and feces | (31) |
| | *Solibacillus* | reduced | mucosa | (25) |
| **Bacteroidetes** | | | | |
| | *Prevotella* | increased | Mucosa and feces | (31) |
| | *Porphyromonadaceae* | increased | feces | (40) |
| | *Bacteroides* | Increased (mucosa and feces) | Mucosa and feces | (31) |
| | *B. dorei* | increased | feces | (61) |
| | *B. massiliensis* | increased | feces | (61) |
| | *Cloacibacterium* | increased | mucosa | (26) |
| | *Lachnospiracae* | reduced | feces | (40) |
| **Actinobacteria** | | | | |
| | *Bifidobacterium* | reduced | Feces and mucosa | (31) |
| **Fusobacteria** | | | | |
| | *Fusobacterium* | increased | Mucosa and feces | (31, 49) |
| **Proteobacteria** | | increased | Mucosa, feces | (25, 68) |
| | *Enterobacteriaceae* | increased | feces | (40) |
| | *Helicobacter* | increased | mucosa | (26) |
| | *Acinetobacter* | increased | mucosa | (26) |
| | *Pseudomonas* | increased | Mucosa, feces | (25, 26, 40) |
| | *Acidovorax* | increased | mucosa | (26) |

## Table 2

| Phylum | Microbiota | Change in CRC compared to control | Type of sample | Reference |
|---|---|---|---|---|
| **Firmicutes** | | increased | mucosa | (62) |
| | *Lactobacillales* | Increased | mucosa | (28) |
| | *Erysipelotrichaceae* | Increased | feces | (28) |
| | *Peptostreptococcus* | increased | rectal swab, feces, mucosa | (28, 32, 35) |
| | *Mogibacterium* | increased | rectal swab | (28) |
| | *Clostridium difficile* | increased | feces | (48) |
| | *Enterococcaceae* | increased | feces | (40, 69) |
| | *Acidaminobacter* | Increased | feces | (70) |
| | *Phascolarctobacterium* | Increased | feces | (70) |
| | *Parvimonas* | increased | mucosa | *(35)* |
| | *Parvimonas micra* | increased | feces | (32) |
| | *Solobacterium moorei* | increased | feces | (32) |
| | *Lactococcus* | increased | mucosa | (62) |
| | *Blautia* | reduced (rectal swab) /increased (mucosa and feces) | rectal swab, mucosa and feces | (28, 31) |
| | *Ruminococcus* | reduced (mucosa, feces), increased (mucosa) | mucosa, feces | (35, 40, 70, 71) |
| | *Roseburia* | Reduced (feces)/increased (mucosa) | feces, mucosa | (30, 35, 69) |
| | *Lactobacillus* | reduced | feces | (69) |
| | *Dorea* | reduced | feces | (70) |
| | *Faecalibacterium* | reduced | mucosa, rectal swab, feces | (28, 31, 69) |
| | *Clostridia* | reduced | feces | (27, 41) |
| | *Anoxybacillus* | reduced | mucosa | (30) |
| **Bacteroidetes** | *Lachnospiracae* | reduced | feces | (41, 70) |
| | *B. ovatus* | increased | feces | (61) |
| | *B. vulgatus* | increased | feces | (61) |
| | *Porphyromonas* | increased | rectal swab, feces, mucosa | (27, 28, 35, 40, 41) |
| | *Prevotella* | reduced (feces), increased (mucosa and feces) | mucosa, feces | (31, 70) |
| | *Parabacteroides* | reduced | mucosa | (71) |

| Phylum | Microbiota | Change in CRC compared to control | Type of sample | Reference |
|---|---|---|---|---|
| **Fusobacteria** | | | | |
| | *Fusobacterium* | increased | rectal swab, mucosa, feces | (27, 28, 31, 32, 35, 40, 41, 48, 62, 69, 71) |
| | *Leptotrichia* | increased | mucosa | (71) |
| **Euryarchaeota** | | | | |
| | *Methanobacteriales* | increased | feces and mucosa | (31) |
| **Proteobacteria** | | reduced | mucosa | (62) |
| | *Citrobacter farmeri* | Increased | feces | (70) |
| | *Escherichia coli* | increased | feces, mucosa | (55, 61, 72) |
| | *Campylobacter* | increased | mucosa, feces | (27, 33) |
| **Verrucomicrobia** | | | | |
| | *Akkermansia muciniphila* | increased | feces | (70) |
| **Actinobacteria** | | | | |
| | *Coriobacteriaceae* | increased | feces | (28) |
| | *Microbacterium* | reduced | mucosa | (30) |
| | *Bifidobacterium* | reduced | rectal swab, feces, mucosa | (28, 31, 61, 72) |

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